The Fine Structure of Kappa in Killer Stock 51 of *Paramecium aurelia*. Preliminary Observations.*

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The killer trait in *Paramecium aurelia* depends upon the presence of a cytoplasmic genetic particle, kappa (1). Animals possessing kappa liberate into the culture medium in which they live a poison, paramecin, to which they themselves are resistant; animals lacking kappa are killed by this substance.

Stained preparations and phase microscope observations of fixed and unfixed kappa reveal, in stock 51, two types of particles: smaller bodies ("non-brights") with no detectable internal structure and larger forms ("brights") associated with paramecin activity and containing a highly refractile body (2). In polar view, this inclusion is circular with a central dark core. In side view, the refractile body usually is square (0.5μ) but occasionally is divided into two equal halves by a dark band (= core) that unites with the kappa matrix at either end of the refractile body. Preer's varied methods of staining the refractile body were unsuccessful (2); but the rest of the kappa particle in whole mount preparations of killer animals was reported to be uniformly Feulgen-positive.

Methods

Killers and sensitives, grown at 27°C., were lightly centrifuged, allowed to recover for 15 minutes and fixed in 1 per cent osmium tetroxide buffered with acetate-veranol to pH 7.2–7.5 for 45 minutes, followed by dehydration and embedding in a mixture consisting of 40 per cent ethyl: 60 per cent *n*-butyl methacrylate polymerized with benzoyl peroxide at 45°C. for 18–24 hours. Duplicate blocks of each type of material were prepared on different days, sectioned with a Porter-Blum microtome and examined in an RCA EMU-2 electron microscope.

OBSERVATIONS

In thin sections, kappa was identified (*a*) as particles present in killer stocks 51 and G and absent in sensitive stocks 51, G, and d4-186, and (*b*) as particles morphologically similar to kappa observed with the ordinary light and phase contrast microscopes.[1]

In the stained killer animal, kappa is distributed through the endoplasm but occasionally is more concentrated near the macronucleus. Fig. 1 is an electron micrograph of kappa in the latter area. Two membranes appear to surround kappa (Fig. 2); the inner one frequently is ill-defined, discontinuous and suggests structural continuity with material inside the kappa particle, including (*a*) a wispy, filamentous component associated with minute "granules" which may be discrete or may represent folding or localized differentiation along the filament, (*b*) small dense particles of irregular shape and (*c*) the refractile body of "brights". The filamentous material usually is more concentrated in the small "non-bright" forms, resulting in their characteristic dense appearance in electron micrographs (Fig. 4). In "brights," on the other hand, the material is much less concentrated and occurs in small aggregates randomly distributed throughout the kappa body (Figs. 1, 4). The filaments are extremely variable morphologically, ranging from delicate, more or less indistinct structures in "non-brights" to coarse, long, branching forms in "brights," frequently anastomosing with finer material.

Small, extremely dense particles of irregular shape are present in both "brights" and "non-brights" but appear with greater frequency in the former. Whether these are distinct from the filamentous material remains uncertain.

Electron micrographs of the refractile body reveal, in a plane corresponding to the polar view (plane A), a series of concentric lamellae, periodically spaced about a central core (Figs. 1, 3). The number of lamellae has been found thus far to vary from three to eleven with indications of a

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[1] An earlier attempt to recognize kappa in electron micrographs was made by Hamilton *et al.* (3), but the identity of kappa was never definitely established. Hamilton's unpublished electron micrographs, interpreted in the light of the current study of the present author and communicated to Hamilton, strongly suggest that his observations were applicable to the "bright" forms, though differences in material and technique resulted in somewhat different images of kappa in the two cases.

direct correlation between size of the "bright" and the number of lamellae comprising the refractile body. The image in Fig. 3 suggests the lamellae are double-membraned structures.

When the plane of section transects the refractile body at right angles to the above and through the region containing the core (plane B) two groups of lamellae are observed, separated by a band of core material. The groups are parallel, directly opposed, and contain an equal number of lamellar profiles terminating abruptly at either end. The refractile body thus appears to be comprised essentially of concentric cylindrical or cylindrically coiled surfaces enclosing a central core of material.

An alternative structural arrangement represented by vertically stacked, flat circular discs of concentric coils could achieve the same general form. If such were the make-up, however, the arrays of even, lamellar profiles found in plane B sections would be expected to be replaced by similarly oriented units individually consisting of either a linear series of transverse profiles of alternating coil and intercoil material, or of transverse profiles of a series of coils packed so closely as to exclude intercoil material. Such configurations have not been observed.

The core of the refractile body is of fairly constant dimension ($\sim 0.26\mu$) and is composed of a concentration of dense granular material. Occasionally, a subspherical to ovoid intracore inclusion body has been observed which, in certain instances, appears to be intimately associated at one point with the innermost lamellar surface. Since Preer has observed that "brights" can originate from "non-brights" (2), it is tempting to consider the core inclusion body as a possible center for the origin of the refractile body.

The relation of kappa to other cell components or organisms is still obscure, and its origin remains unknown. Certainly, the earlier suggestions that related it to virus-like particles or to *Chlorella*, the green algae symbiont in *Paramecium bursaria* (4) now appear untenable in view of our present knowledge of kappa structure. Attention has also been directed to similarities between the lamellar configurations of the refractile body and those of mitochondria, chloroplasts, concentric lamellae of the cortical granules of *Arbacia* oocytes (6) and bodies in the proximal renal tubules of young mice (7), etc. Most of these comparisons are of limited value, since they frequently relate structures whose dimensions are of a different order of magnitude and deal only with the refractile body without regard for its relation to the remainder of the kappa particle. On the other hand, electron micrographs do reveal a number of similarities between kappa and certain rickettsial and bacterial forms (8, 9). (The possible relation between kappa and rickettsiae was suggested earlier (5) by Preer) Kappa apparently can still be distinguished from these organisms by one or more criteria (most notably that of differences in DNA distribution based on Feulgen-stained material). However, the above observations, considered together with more general evidences of similarity (*e.g.*, infective capacity, etc.) suggest that an attempt to relate kappa to other living forms might profitably include a critical cytological and cytochemical comparison of the above organisms, especially those symbiotic groups known to produce exotoxins and to contain inclusion bodies associated with toxic activity.

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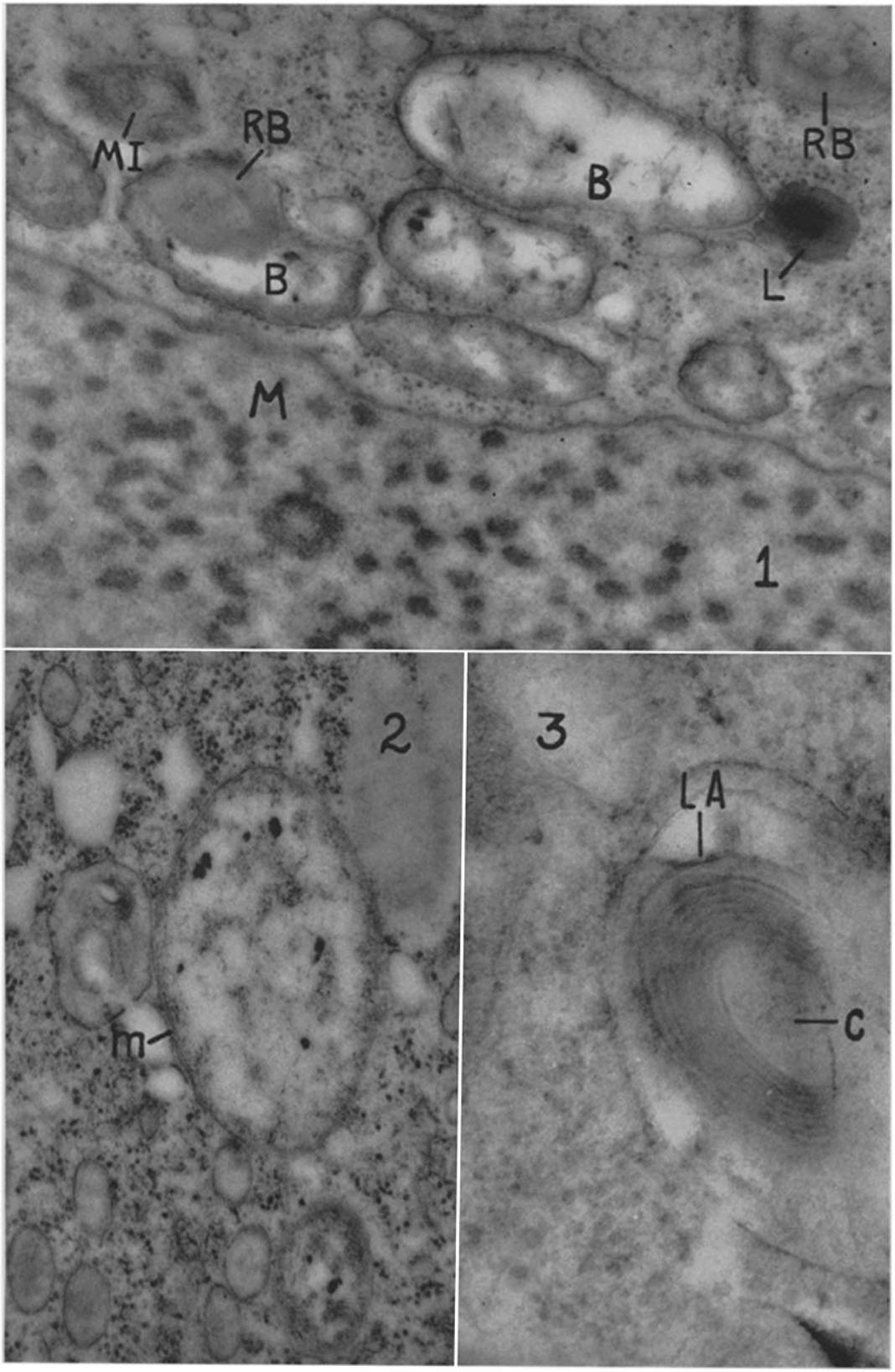
Explanation of Plates

PLATE 59

FIG. 1. Section showing numerous kappa bodies adjacent to the macronucleus, *M*. *B*, "bright" kappa body, associated with paramecin activity; *RB*, refractile body within "brights." Note the presence of irregular, coarse filamentous material in the "brights" and the tendency for this and finer material to be more concentrated at the periphery of the body. Note also the small dense particles in the kappa matrix. *L*, lipide body; *MI*, mitochondrion. Approximately × 32,500.

FIG. 2. A "bright" kappa body showing evidence of two limiting membranes, *m*. Approximately × 38,500.

FIG. 3. A slightly oblique section through the refractile body of a "bright" kappa particle. The series of concentric lamellae (*LA*) typically found in transverse sections through refractile bodies is clearly evident. *C*, core material.

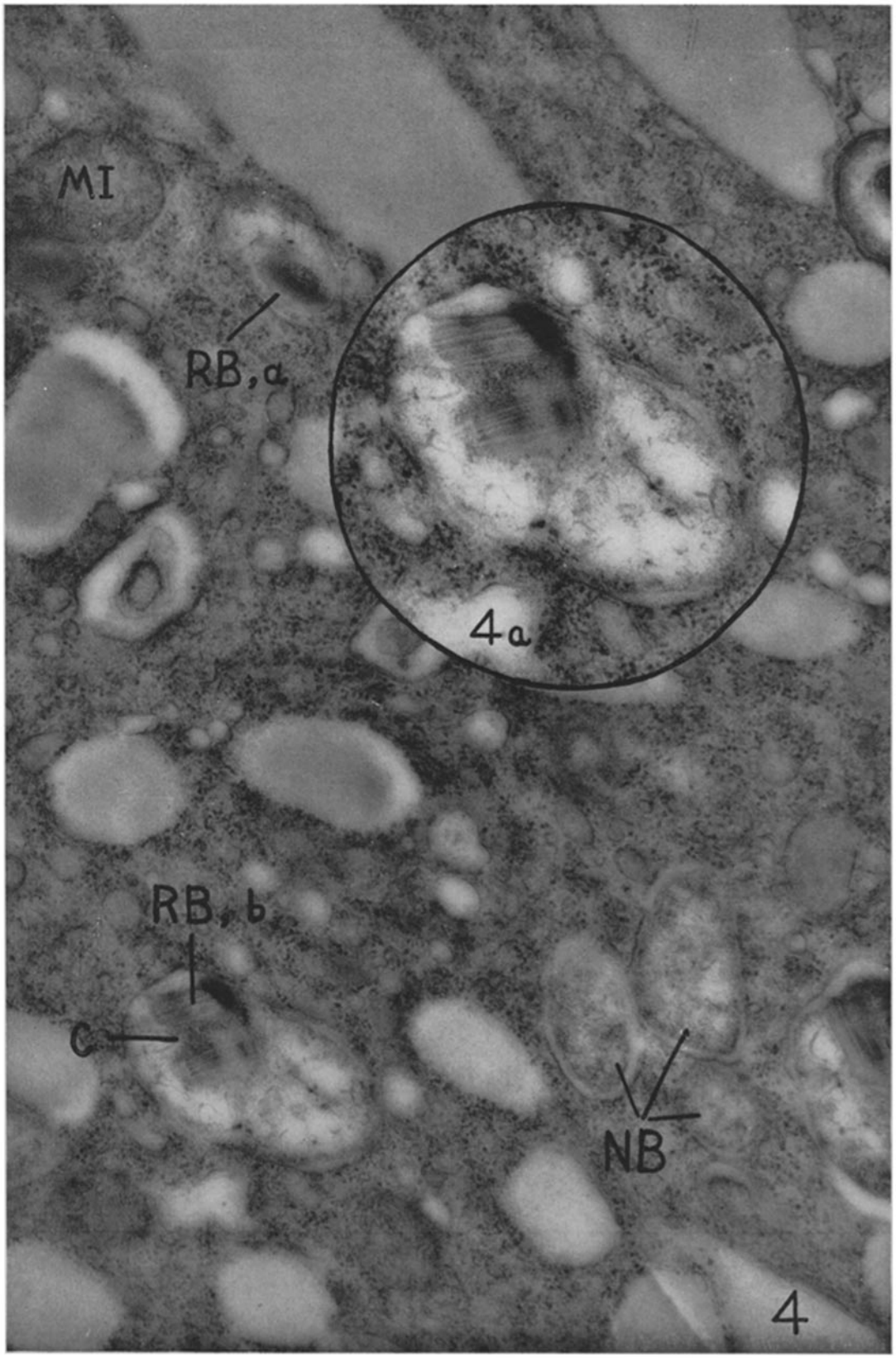


(Dippell: Structure of kappa in *Paramecium aurelia*)

PLATE 60

FIG. 4. Section through a cytoplasmic area containing numerous kappa bodies. Refractile bodies appear in two diverse planes of section: *a*, plane similar to that in Figs. 1 and 3; *b*, plane perpendicular to *a*. Note the greater concentration of material in the smaller ("non-bright") kappa bodies, *NB*, in contrast with that in the "bright" forms. Approximately × 25,800.

FIG. 4 *a*. "Bright" enlarged from Fig. 4. × 38,500.



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